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Supplemental Material

Biochemical properties and crystal structure of a novel β-phenylalanine aminotransferase from *Variovorax paradoxus*

Ciprian G. Crismaru,[a,#] Gjalt G. Wybenga,[b,#] Wiktor Szymanski,[c] Hein J. Wijma,[a] Bian Wu,[a] Sebastian Bartsch,[a] Stefaan de Wildeman,[d] Gerrit J. Poelarends,[e] Ben L. Feringa,[c] Bauke W. Dijkstra[b] and Dick B. Janssen[a,*]

[a]*Department of Biochemistry, and* [b]*Laboratory of Biophysical Chemistry, Groningen Biomolecular Science and Biotechnology Institute,* [c]*Center for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, the Netherlands,* [d]*DSM Pharmaceutical Products, Geleen, The Netherland,* [e]*Department of Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, the Netherlands*

[#] These two authors contributed equally to this study.

* For correspondence: E-mail: d.b.janssen@rug.nl; Tel: (+31) 50-3634008; Fax: (+31) 50-3634165; Address: Nijenborgh 4, 9747 AG Groningen, The Netherlands.

Supplemental Material Table S1

TABLE S1. Purification of the recombinant *Vp*AT from *E. coli* strain C41(DE3).

Purification step	Sp. activity ($U \cdot mg^{-1}$)	Total protein (mg)	Total activity (U)	Purification (fold)	Recovery (%)
Cell-free extract	2.9	1134	3288	1	100
HisTrap chromatography	7.4	248	1835	2.5	56
Q-Sepharose chromatography	17.5	90.4	1582	6	48

Supplemental Material Table S2

TABLE S2 Data collection and refinement statistics of *Vp*AT crystal structures.

Parameters	*Vp*AT holo	*Vp*AT + AOA
Beamline	ID14-4	ID14-4
Data collection		
Resolution (Å) [a]	46.42-1.5 (1.58-1.5)	66.31-2.28 (2.4-2.28)
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, b, c (Å)	88.74, 100.1, 104.8	88.67, 99.88, 104.7
R_{merge} [b]	0.043 (0.136)	0.095 (0.158)
Wavelength (Å)	0.9395	0.9395
$I/\sigma(I)$	21.9 (9.3)	10.9 (7.5)
Completeness (%)	99.7 (100.0)	99.8 (99.8)
No. unique reflections	148964 (21628)	43283 (6220)
Multiplicity	4.8	4.0
Refinement		
Resolution (Å)	1.5	2.3
R_{work} [c]	0.17	0.18
R_{free} [d]	0.18	0.23
Rmsd		
Bond lengths (Å)	0.08	0.08
Bond angles (°)	1.22	1.11
Ramachandran (Molprobity)		
Favored (%)	98	97.0
Outliers (%)	0.0	0.0
PDB code	4AO9	4AOA

[a] Values in the parentheses are for the highest resolution shell.

[b] $R_{merge} = \Sigma_h\Sigma_i |I(h)_i - \langle I(h)\rangle| / \Sigma_h\Sigma_i \langle I(h)_i\rangle$, where I is the observed intensity and $\langle I\rangle$ is the average intensity of multiple observations of symmetry-related reflections.

[c] $R_{work} = \Sigma_h||F_{obs}| - |F_{calc}|| / \Sigma_h|F_{obs}|$, where $|F_{obs}|$ and $|F_{calc}|$ are the observed and calculated structure factor amplitudes, respectively.

[d] R_{free} is calculated as R_{work} using 5 % of all reflections randomly chosen and excluded from structure calculation and refinement.

Supplemental Material Table S3

TABLE S3 List of putative aromatic β-amino acid aminotransferases.

	NCBI reference sequence	annotation	strain	seq. id.
1	CCF34891	aminotransferase class-III	*Colletotrichum* higginsianum	41
2	EFQ30679	aminotransferase class-III	*Glomerella graminicola* M1.001	41
3	EFW16762	conserved hypothetical protein	*Coccidioides posadasii* str. Silveira	39
4	EFW99062	aminotransferase class 3	*Grosmannia clavigera* kw1407	38
5	EFZ00111	glutamate-1-semialdehyde 2,1-aminomutase, putative	*Metarhizium anisopliae* ARSEF 23	42
6	EGU77831	hypothetical protein FOXB_11695	*Fusarium oxysporum* Fo5176	44
7	EGX43269	hypothetical protein AOL_s00215g5	*Arthrobotrys oligospora* ATCC 24927	38
8	EGX48176	hypothetical protein AOL_s00081g39	*Arthrobotrys oligospora* ATCC 24927	42
9	EGY14282	glutamate-1-semialdehyde 2,1-aminomutase	*Verticillium dahliae* VdLs.17	41
10	EHA25332	hypothetical protein ASPNIDRAFT_186860	*Aspergillus niger* ATCC 1015	37
11	EHK26440	hypothetical protein TRIVIDRAFT_36134	*Trichoderma virens* Gv29-8	43
12	EHK45279	hypothetical protein TRIATDRAFT_138933	*Trichoderma atroviride* IMI 206040	40
13	EIM84416	aminotransferase class-III	*Stereum hirsutum* FP-91666 SS1	49
14	GAA86562	aminotransferase class-III	*Aspergillus kawachii* IFO 4308	37
15	GAA93170	aminotransferase class-III	*Aspergillus kawachii* IFO 4308	37
16	XP_001218204	predicted protein	*Aspergillus terreus* NIH2624	37
17	XP_001248198	hypothetical protein CIMG_01969	*Coccidioides immitis* RS	40
18	XP_001824703	acetylornithine aminotransferase	*Aspergillus oryzae* RIB40	41
19	XP_001930822	glutamate-1-semialdehyde 2,1-aminomutase	*Pyrenophora tritici-repentis* Pt-1C-BFP	39
20	XP_002145750	glutamate-1-semialdehyde 2,1-aminomutase, putative	*Penicillium marneffei* ATCC 18224	33
21	XP_002150372	glutamate-1-semialdehyde 2,1-aminomutase, putative	*Penicillium marneffei* ATCC 18224	43
22	XP_002383909	acetylornithine aminotransferase, putative	*Aspergillus flavus* NRRL3357	41
23	XP_002483365	acetylornithine aminotransferase, putative	*Talaromyces stipitatus* ATCC 10500	38
24	XP_003040355	hypothetical protein NECHADRAFT_34859	*Nectria haematococca* mpVI 77-13-4	39
25	XP_003065334	aminotransferase class III family protein	*Coccidioides posadasii* C735 delta SOWgp	39
26	XP_003295515	hypothetical protein PTT_01400	*Pyrenophora teres f. teres* 0-1	39
27	XP_003711910	glutamate-1-semialdehyde 2,1-aminomutase	*Magnaporthe oryzae* 70-15	39
28	XP_381362	hypothetical protein FG01186.1	*Gibberella zeae* PH-1	42
29	YP_001862264	class III aminotransferase	*Burkholderia phymatum* STM815	55
30	YP_001890102	class III aminotransferase	*Burkholderia phytofirmans* PsJN	58
31	YP_002944294	class III aminotransferase	*Variovorax paradoxus* S110	87
32	YP_003481853	aminotransferase class-III	*Natrialba magadii* ATCC 43099	35
33	YP_004154921	class III aminotransferase	*Variovorax paradoxus* EPS	87
34	YP_004978979	class III aminotransferase	*Burkholderia sp.* YI23	56
35	YP_006397172	glutamate-1-semialdehyde 2,1-aminomutase	*Sinorhizobium fredii* USDA 257	43
36	ZP_02187576	aminotransferase class-III	*alpha proteobacterium* BAL199	50
37	ZP_02885261	aminotransferase class-III	*Burkholderia graminis* C4D1M	56
38	ZP_06895587	possible glutamate-1-semialdehyde 2,1-aminomutase	*Roseomonas cervicalis* ATCC 4995	42
39	ZP_07281103	glutamate-1-semialdehyde 2,1-aminomutase	*Streptomyces* sp. AA4	41
40	ZP_07777755	aminotransferase class-III	*Pseudomonas fluorescens* WH6	52
41	ZP_08528159	threonine dehydratase	*Agrobacterium* sp. ATCC 31749	27
42	ZP_09395248	glutamate-1-semialdehyde 2,1-aminomutase	*Acetobacteraceae bacterium* AT-5844	50
43	ZP_10156387	class III aminotransferase	*Hydrogenophaga* sp. PBC	50
44	ZP_10393152	glutamate-1-semialdehyde aminotransferase	*Acidovorax* sp. CF316	56
45	ZP_10477025	class III aminotransferase	*Pseudomonas* sp. Ag1	54
46	ZP_10571586	glutamate-1-semialdehyde aminotransferase	*Variovorax* sp. CF313	90

Seq. id. = percentage sequence identity to the protein sequence of *Vp*AT.

Supplemental Material Fig. S1

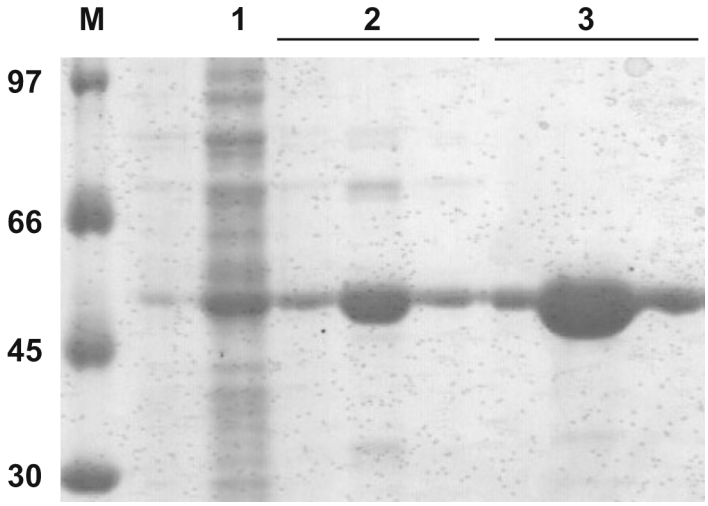

FIG S1. SDS-PAGE of *Vp*AT at different steps of purification. The gel contained 10% polyacrylamide. Lanes: M, molecular weight marker (kDa); 1, cell-free extract of *E. coli* strain C41(DE3) expressing the recombinant enzyme; 2, HisTrap fraction; 3, Q-Sepharose HP fraction.

Supplemental Material Fig. S2

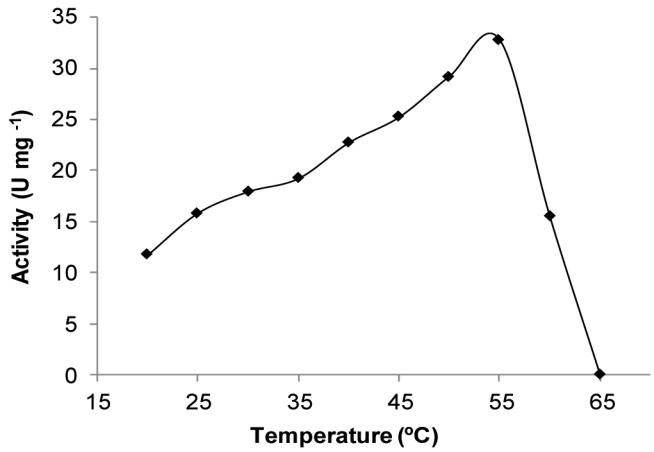

FIG S2. Effect of temperature on the specific activity of *Vp*AT.